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U. S. DEPARTMENT OF AGRICULTURE.
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.

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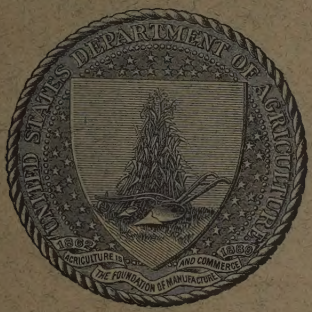
BORDEAUX MIXTURE:

ITS

CHEMISTRY, PHYSICAL PROPERTIES, AND TOXIC EFFECTS ON FUNGI AND ALGÆ.

BY

WALTER T. SWINGLE,
ASSISTANT, DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.



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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY,
Washington, D. C., March 20, 1896.

SIR: I have the honor to transmit herewith a technical bulletin on the chemistry and physical properties of bordeaux mixture, together with a summary of our present knowledge on the toxic effects of this preparation on fungi and algæ. The bulletin has been prepared by Mr. Walter T. Swingle, an assistant in this division, the matter being based upon laboratory and field work carried on in 1894. The object of the bulletin is not so much to record the facts brought out by the investigations as to suggest desirable lines of work that might be undertaken by experiment station workers and others. There are many problems—chemical, physical, and biological—connected with this truly remarkable fungicide that remain to be solved, and it is hoped that the suggestions in the accompanying pages will be useful in bringing about their solution.

Respectfully,

B. T. GALLOWAY,
Chief of Division.

Hon. J. STERLING MORTON,
Secretary.

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BORDEAUX MIXTURE: ITS CHEMISTRY, PHYSICAL PROPERTIES, AND TOXIC EFFECTS ON FUNGI AND ALGÆ.

INTRODUCTION.

Bordeaux mixture, although known but a decade, has come to be widely used as a fungicide. In France, where it originated, and more especially in America, it is used with great success in preventing many fungous diseases of plants. This mixture, made by neutralizing a solution of copper sulphate with milk of lime, is quite complex in its chemical composition and physical structure. Slight variations in the relative amounts of its constituents and in the manner of mixing them have an astonishingly great effect on the nature of the preparation and its effectiveness as a fungicide. It is therefore very important to understand what constitutes properly made bordeaux mixture in order that the less effective forms may be avoided.

Since the copper hydroxide present in the dried deposit on the leaves is now considered to be the active agent in preventing the attacks of parasitic fungi, and as it probably acts only when dissolved, the conditions governing solubility are reviewed at some length. To a considerable extent these same conditions govern the solubility of the copper salts present in other fungicides. It is hoped that this part of the article may prove of general interest to botanists.

Notwithstanding the widespread use of bordeaux mixture, the exact nature of its action in preventing the ravages of parasitic fungi has been but little studied and is still only imperfectly understood. Its action is one of very great importance in practice, and is, moreover, of considerable interest to physiologists. In this bulletin an attempt is made to present a summary of our present knowledge on the subject, and with this end in view all literature relating to the amount of copper necessary to kill fungi and algæ has been collected and briefly summarized. Nägeli's very remarkable results regarding the action of extremely minute amounts of metallic copper and copper salts in poisoning algæ are so suggestive that they have been abstracted at some length. It is important to know the exact stage of development of parasitic fungi when they are poisoned by copper contained in bordeaux mixture, since it explains the high preventive and curative action of the preparation.

Finally, a number of provisional hypotheses are suggested to explain the observed toxic action of bordeaux mixture on parasitic fungi. These suggestions are made principally with a view of stimulating further inquiry in this line. Throughout the bulletin matters of purely historical interest have been purposely avoided, the effort being made to present, in as logical a manner as possible, the results of the really important studies bearing on the matter under consideration.

The questions considered are discussed under the following heads: (1) Chemical composition of bordeaux mixture; (2) physical nature, cause of settling; (3) conditions governing the adherence of the mixture and the solubility of copper; (4) amounts of copper in solution necessary to kill fungi and other cryptogams; (5) stage of development of parasitic fungi when acted on, and how entrance into the host plant is prevented; (6) hypothesis as to the action of copper in poisoning fungi.

CHEMICAL COMPOSITION OF BORDEAUX MIXTURE.

As is well known, bordeaux mixture is made by stirring together a solution of copper sulphate ($CuSO_45H_2O$) and milk of lime ($Ca(OH)_2$). When freshly prepared, the resulting sky-blue mixture consists essentially of a nearly or quite saturated aqueous solution of calcium sulphate and calcium hydrate, containing in suspension a considerable quantity of calcium sulphate and copper hydroxide and also a varying amount of calcium hydroxide. Undoubtedly a very small amount of calcium carbonate is also found in suspension, since all ordinary lime contains it as an impurity, and the limewater by absorbing carbonic acid from the air forms small quantities on standing, as does indeed, to a less degree, the finished mixture. Very possibly certain basic sulphates of copper occur in suspension in all ordinary bordeaux mixture. These basic sulphates certainly abound when an insufficient quantity of lime is used. Their presence, when they exist in considerable quantity, is shown by the greenish blue color of the mixture.

Greater or less variations in the composition of the mixture may be caused by impurities present either in the copper sulphate or in the lime. Copper sulphate is likely to contain small amounts of iron as sulphate, which, in making bordeaux mixture, would be changed to iron hydrate upon adding lime. Commercial lime contains more or less impurities, and these vary in nature according to the composition of the limestone burned in making the lime. Usually silica and small amounts of salts of aluminum and magnesium are found in the mixture as a result of the use of ordinary lime. The exact nature of the impurities in lime and their effects on the composition of bordeaux mixture have been studied but little. It should be noted, however, that by far the most soluble constituent of properly prepared bordeaux mixture is calcium sulphate.

According to various authors, the quantity of water necessary to dissolve 1 part of calcium sulphate is:

At—		Parts water.	At—		Parts water.
Degrees C.	Degrees F.		Degrees C.	Degrees F.	
0	32	[1] 525	40	104	[4] 467
5	41	[2] 541	41	105.8	[1] 468
15.2	59.5	[3] 543	53	127.4	[1] 474
18	64.4	[4] 588	60	140	[4] 481
20	68.2	[5] 485	72	161.6	[1] 495
24	75.2	[1] 479	80	176	[4] 513
32	89.6	[1] 470	86	186.8	[1] 528
35	95	[6] 394	90	210.2	[1] 571
38	100.4	[1] 466	100	212	[4] 575

[1] *Marignac.* Ann. Chim. Phys., 15, 1874, p. 279, as quoted by E. Wiedemann, Lösungen, Ladenburg, Handwörterb. d. Chem., 6, p. 533.

[2] *Wiedemann*, l. c., Ladenburg, 6, 553. Calculaetd from Raupenstrauch's formula (for $CaSO_4$ from 0° to 32°, $S=0.1771+0.00187162\ (t-0.8)-0.04247095\ (t-0.8)^2$).

[3] *Holleman, A. F.* Die Bestimmung der Löslichkeit einiger sogenannter unlöslicher Salze (Maandbl. Naturw., 18, pp. 45–58; according to review in Chem. Centralbl., 44, II, 1893, p. 521).

[4] *Marignac.* Ann. Chim. Phys., 5, I, p. 274 (quoted by R. Biedermann, Calcium, Ladenburg, Handwörterb. d. Chem., 2, 443; original not seen).

[5] *Kohlrausch, Fr.*, and *Rose, Fr.* Die Löslichkeit einiger schwerlöslicher Körper im Wasser, beurteilt aus der elektrischen Leitungsfähigkeit der Lösungen (Math.-Naturw. Mitt. a. d. Sitzungsb. d. kgl. pr. Akad. d. Wissensch. zu Berlin, 1893, pp. 257–284; according to review in Chem. Centralbl., 44, II, 1893, p. 466).

[6] *Poggiale*, quoted in Roscoe, H. E., and Schorlemmer, C., Ausführliches Lehrb. der Chem., 2, p. 163.

According to Poggiale,[1] the temperature of maximum solubility of this salt is about 35° C. One liter of concentrated solution would contain about 1.8 grams of $CaSO_4$ at 15.2°, 2.54 grams at 35°, and 1.74 grams at 100° C. The presence of sulphates in solution can be easily proved by allowing the bordeaux mixture to stand until the supernatant liquid is perfectly clear, and then adding a few drops of a solution of barium chloride. A milky white precipitate is at once formed, and this will not dissolve in hydrochloric acid. Another test is to acidulate the clear liquid with acetic acid and then add strong alcohol in considerable excess. A pale white precipitate indicates the presence of calcium sulphate, which is nearly insoluble in strong alcohol. Calcium hydroxide is also present in saturated solution in bordeaux mixture as usually prepared, i. e., when an excess of lime is used. This substance is more soluble in cold than in hot water, as will be seen from the following table from Th. Maden[2] showing the number of parts of water at different temperatures required to dissolve 1 part of CaO:

At—		Parts water.	At—		Parts water.
Degrees C.	Degrees F.		Degrees C.	Degrees F.	
0	32	763	40	104	934
5	41	769	50	122	1,020
10	50	781	60	140	1,136
15	53	787	70	158	1,250
20	68	794	80	176	1,370
25	77	833	90	194	1,587
30	86	862	99	210.2	1,667

[1] *Poggiale, l. c.*

[2] *Maden, Th.* Pharm. Jour. and Trans. (quoted from Wiedemann, Lösungen, Ladenburg, Handwörterb. d. Chem., 6, 533).

It will be readily seen that hot, recently slacked milk of lime must contain much less calcium hydroxide in solution than when cooled after standing. This may in part account for the different action of the reaction when hot milk of lime and copper sulphate solution are mixed. It is very advantageous to have both constituents cool before mixing.

The figures given above do not as a matter of fact represent the real capacity of the supernatant liquid of bordeaux mixture, since both calcium sulphate and calcium hydroxide are in solution at once, and hence the hydroxide, for instance, must be considered as dissolving not in pure water, but in a concentrated[1] or nearly concentrated solution of calcium sulphate. Doubtless the figures given for the solubility in water do not exactly represent the solubility under these conditions. From the nature of the two salts it is, however, probable that they affect each other but little in dissolving.

Calcium carbonate is a third salt which is also present in suspension in almost all bordeaux mixture, even when freshly made, and doubtless gradually dissolves until it forms a concentrated solution. R. Biedermann[2] says that to dissolve 1 part of calcium carbonate requires 16,600 parts of cold and 8,860 parts of hot water, while C. R. Fresenius[3] says, on the authority of Hofmann and Weltzen, that by protracted boiling, 1 part of $CaCO_3$ requires 28,500 parts of water. The most recent investigations, however, give the quantity of water necessary to effect a solution as much larger. According to A. F. Hollemann,[4] at 23.8° C. 80,040 parts are needed. Fr. Kohlrausch and Fr. Rose[5] give the solubility of calcite at 18° C. as 13 milligrams per liter, or 1 part in 76,923 parts water, while the heavier and harder arragonite dissolves in 66,667 parts water, or 15 milligrams per liter. Unlike calcium hydrate, the

[1] As the copper sulphate in solution doubtless precipitates the hydroxide as fast as it dissolves, when the reaction ceases it is highly probable that the calcium hydroxide is not present in concentrated solution. On the other hand, calcium sulphate is being produced in a nascent state all through the liquid, and doubtless is present in nearly saturated solution when the chemical action stops. Moreover, the heat developed by the reaction would aid the sulphate to dissolve (up to 35° C.) and at the same time hinder solution of the hydrate. As the solution cools it will hold less and less sulphate and more and more hydrate. It is therefore justifiable to speak of hydrate dissolving in a strong solution of sulphate.

[2] *Biedermann, R.* Calcium, Ladenburg, Handwörterb. d. Chem., 2, 1884, p. 448.

[3] *Fresenius, C. R.* A System of Instructions in Quantitive Chemical Analysis (from the last English and German editions; edited by O. D. Allen, with the cooperation of Samuel W. Johnson. John Wiley & Sons, New York, 1890, p. 144).

[4] *Hollemann, A. F.* Die Bestimmung der Löslichkeit einiger sogenannter unlöslicher Salze (Zeit. f. Phys. Chem., Bd. XII, 1893, pp. 125–139; see also Maandbl. Naturw., 18, pp. 45–48; quoted from review in Chem. Centralbl., 44, 1893, II, No. 10, p. 521).

[5] *Kohlrausch, Fr.*, and *Rose, Fr.* Die Löslichkeit einiger schwerlöslicher Körper im Wasser, beurteilt aus der elektrischen Leitungsfähigkeit der Lösungen (Math.-Naturw. Mitt. a. d. Sitzungsb. d. kgl. pr. Akad. d. Wissensch. zu Berlin, 1893, pp. 275–284; review in Chem. Centralbl., 44, II, 1893, p. 466).

carbonate is more soluble in cold water, while, as has been seen before, the liquid contained in the bordeaux mixture soon becomes a saturated solution of calcium sulphate and hydrate. According to Hunt,[1] normal calcium salts increase the solubility of calcium carbonate, and doubtless it dissolves more readily in the bordeaux mixture than in water. Water containing carbonic acid in solution dissolves calcium carbonate much more readily than pure water,[2] but as there is an excess of calcium hydrate in bordeaux mixture and as this compound seizes carbonic acid with avidity it is highly probable that there is no free carbonic acid in solution. It is only after the mixture dries on the leaves and the hydrate is neutralized by the carbonic acid of the air in solution in rain and dew that the solvent action of water containing carbon dioxide can be exerted on the calcium carbonate.

Copper hydrate is said by Storer[3] to be insoluble in water. Millardet and Gayon[4] found that until the calcium hydrate had been neutralized by carbonic acid from the air none of the copper in bordeaux mixture, when the latter was spread on filter paper and dried, was dissolved until after from one to ten days' exposure. It is therefore very probable that properly prepared bordeaux mixture has no copper whatever in solution. Little is known as to the conditions governing solubility of the basic copper sulphates present in improperly made bordeaux mixture.

Of the matter in suspension in the freshly prepared mixture, calcium sulphate probably constitutes the largest amount by weight, but the copper hydrate is more bulky; the former is in the shape of small granules or crystals, while the latter is in the form of thin precipitation membranes, as will be seen later. A considerable quantity of calcium hydroxide is to be found in suspension in ordinary bordeaux mixture, and small amounts of calcium carbonate, which substances constantly increase as the mixture stands. More or less of basic sulphates of copper also are likely to be found suspended, especially in improperly made bordeaux mixture.

Upon drying and exposure to the action of the air and rain or dew, any excess of calcium hydroxide originally present in the mixture becomes neutralized by carbonic acid, and calcium carbonate is formed. Very possibly a similar change is sometimes undergone by the copper hydrate, but this is not as yet established.

The exact sequence of chemical changes in the course of preparation of the mixture is as yet not at all well worked up. The fact that one

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[1] Quoted by C. R. Fresenius, l. c., p. 144.

[2] According to Roscoe and Schorlemmer (Ausführliches Lehrb. der Chem., 2, 1879, p. 167), at 0° C., 1 part dissolves in 1,429 parts of water saturated with carbon dioxide at ordinary pressure (1 liter containing 0.70 grams), and at 10° C. 1 part dissolves in 1,136 of water (1 liter containing 0.88 grams).

[3] *Storer, Frank.* Dictionary of Chemical Solubilities, 1864, p. 440.

[4] *Millardet et Gayon.* Les divers procédés de traitement du mildiou par les composés cuivreux (Jour. d'Agr. prat., 51e ann., T. I, No. 20, May, 1887, p. 701).

of the two components, calcium hydrate, is almost all in a solid state when added, renders it very probable that the amount of copper sulphate in solution greatly exceeds that of calcium hydrate, at least in the earlier stages of the reaction. In case the amount of milk of lime used is insufficient to fully neutralize the copper sulphate, the resulting mixture shows a greenish blue color; this is doubtless due to the presence of some of the numerous basic sulphates of copper. If more lime is added, generally this greenish color of the precipitate slowly changes into a deep sky blue, doubtless because of the basic sulphates of copper being changed to copper hydroxide. When there is a slight excess of milk of lime and both the copper sulphate solution and the milk of lime have been diluted, this deep blue color is assumed almost instantly. It is possible that even here basic sulphates are first formed and then very quickly changed to hydrate of copper.

Of the chemical changes undergone by the mixture on drying and exposure to the air and rains, the first and most important is the absorption of carbon dioxide by calcium hydroxide, forming calcium carbonate. It is probable that in dry air little of such absorption takes place, but in damp air, and especially when rain or dew containing CO_2 in solution wets the lime, the action is rapid. Eventually all the calcium hydroxide is neutralized—a process, according to Millardet and Gayon,[1] requiring, even when there are abundant rains, from a week to ten days in case the bordeaux mixture is rich in lime, and some little time for a mixture having as little lime as possible. After the neutralization of the calcium hydroxide is completed, rain water containing CO_2 in solution is able to dissolve the calcium carbonate and gradually wash it off.

It is not known whether any change is undergone by the copper hydroxide. Undoubtedly no change takes place until all the calcium hydroxide is neutralized. From some results obtained in recent field work the writer suspects that under the influence of carbonic acid, especially when in solution in rain or dew, the copper hydroxide is sometimes changed to basic carbonate.[2] Investigations on this point are now in progress. After the lime is neutralized the small amount of ammonia and nitric and nitrous acids contained in meteoric waters act on and dissolve small amounts of the copper salts. The chemical changes, if

[1] *Millardet et Gayon.* La bouillie bordelaise céleste (Jour. d'Agr. prat., 54e ann., T. I, No. 8, Feb. 20, 1890, p. 272).

[2] In his bulletin on bordeaux mixture, Mr. D. G. Fairchild suggests that the basic copper sulphates when subjected to the influence of carbonic acid, break up into normal sulphate and basic carbonate. The injury observed from a mixture containing basic sulphate is said to occur only after a lapse of some little time after rains have fallen on the sprayed foliage. The writer would suggest that the cause of this delay in the appearance of the injury is that no carbonic acid can act on the basic sulphate while calcium hydroxide is still present, because of the much greater affinity of the latter for the CO_2. As soon as neutralization of the lime is completed the carbonic acid can attack the basic sulphate.

any, accompanying this solution have not as yet been studied. The fungicidal action of the mixture is doubtless due to these small amounts of copper in solution, as will be seen later.

PHYSICAL PROPERTIES OF BORDEAUX MIXTURE.

When a moderately diluted milk of lime is mixed with a solution of copper sulphate which is not too dilute, an abundant precipitate is at once formed. Microscopic examination reveals the interesting fact that this precipitate is largely made up of precipitation membranes, often in the form of small cells. The membranes are exceedingly thin, and doubtless this is the cause of the precipitate being very bulky and slow in settling. Besides the bluish membranes, there is usually an amorphous granular precipitate, partly adhering to the membranes and partly free in suspension in the liquid. The precipitation membranes that are closed show very curious phenomena of growth. The membrane breaks at some point, probably because of pressure from within, and as the solution pours out of the break a new membrane is at once formed, inclosing the liquid a second time. This process is often repeated several times, until finally the cell either collapses or the composition of the liquid it contains becomes the same as that without. The great majority of the membranes seen in the freshly prepared mixture are not closed. This is probably because the calcium hydroxide solution is necessarily very weak.[1] Frank Schwarz[2] states that copper acetate solution of 0.5 per cent or less and potassium ferrocyanide of 0.4 per cent or less give no complete cells. Even where a strong solution (8 per cent) of copper acetate was used, a 0.4 per cent potassium ferrocyanide solution gave no homogeneous membrane. It is very advantageous that the solution of calcium hydrate can not be made more concentrated, since it tends to prevent the formation of thick and difficultly permeable membranes, such as often occur where two very strong solutions are mixed.

The writer has found that bordeaux mixture prepared from diluted solutions settles more slowly and the chemical action also seems to be accomplished more quickly. This, it is believed, may be because in a dilute solution fewer closed cells are formed which serve to delay the reaction. It is especially disadvantageous to use lime containing coarse granules, for when once inclosed in a precipitation membrane the process of solution and precipitation is greatly delayed. The attention of the writer was first drawn to the matter when attempting to prepare bordeaux mixture neutral in reaction. Some poorly slacked granular lime was used and it was found in a number of cases that after standing twelve to twenty-four hours the mixture became distinctly alkaline, although neutral when prepared.

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[1] See table of solubility on page 9.

[2] *Schwarz, Frank.* Die morphologische und chemische Zusammensetzung des Protoplasmas; 28 Fällungserscheinungen und künstliche Strukturen (Beiträge zur Biologie der Pflanzen, herausg. v. F. Cohn, 5, H. 1, p. 147).

Some precipitation membranes are impermeable to various solutions. It is not known, however, to what extent the membranes formed in bordeaux mixture are permeable to calcium hydroxide or copper sulphate, and the subject offers a promising field for research.

In any attempts to make a mixture similar to bordeaux, but using potassium or sodium hydroxide instead of lime, the effect of the concentration of the solution on the character of the precipitation membranes would be much greater, since these hydrates are easily obtained in very concentrated solutions.[1]

It is probable that the freshly precipitated copper hydroxide is in the colloid state and contains much water. This would increase the bulk, reduce the specific gravity of the membranes, and greatly impede settling. To account for the changes undergone by the mixture on standing, it seems necessary to assume that the molecules in the membranes are in a labile arrangement. The extremely bulky nature of the fresh precipitate would also be explained if it could be assumed that it is distended with imbibed water.

The calcium sulphate is apparently never thrown down as precipitation membranes, but always as minute granules or small crystals. The specific gravity of the hydrated sulphate ($CaSO_4 2H_2O$) is from 2.2 to 2.4.[2] Because of its low specific gravity and very fine state of subdivision it settles slowly.

After standing, very interesting changes take place in the precipitates of the mixture. After a few days, especially if the mixture is agitated occasionally, the precipitates become very much less bulky, and microscopic examinations show that instead of delicate precipitation membranes of $Cu(OH)_2$ and granules of $CaSO_4 2H_2O$ the deposit consists of beautiful, regular, and good-sized crystals. In many cases the copper hydroxide is in the form of splendid blue sphærocrystals, while the gypsum is usually in twin crystals. It has puzzled the writer to account for the formation of crystals of copper hydroxide, since it is almost absolutely insoluble in the fluid in which it is suspended. However, it is thought that the fresh precipitate in its colloid condition imbibes a considerable quantity of water without dissolving, and may allow the molecules sufficient lability to enable them to rearrange themselves in the form of crystals.[3] As to the increase in

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[1]The writer would suggest that the difficulty experienced by chemists in washing out the alkali from precipitated copper hydroxide may be due to the formation of cells having a thick membrane and imprisoning the alkali. Possibly the difficulty might be avoided by using more dilute solutions in precipitating.

[2]*Biedermann, R.* Calcium, Ladenburg, Handwörterb. d. Chem., 2, p. 442.

[3]*Schwarz, Frank.* Die morphologische und chemische Zusammensetzung des Protoplasmas (Beiträge zur Biologie der Pflanzen, herausg. v. F. Cohn, Heft. 5, I, pp. 140–154). The changes occurring during the slow growth and on the standing of precipitation membranes of copper ferrocyanide and other compounds are described and illustrated. In a number of cases a gradual growth of very small granules or crystals within the membrane is described and some such changes are figured.

size of the crystals of gypsum, all the writer can suggest is that as the solution cools after being warmed by the heat liberated in the double composition, it becomes saturated and still more gypsum crystallizes.

When sprayed upon the foliage, the drops of bordeaux mixture soon begin to show crystals. A pellicle of calcium carbonate quickly forms and as evaporation proceeds calcium sulphate and probably some calcium hydrate crystallize. As long as wet, and whenever wetted with rain or dew, the neutralization of the caustic lime proceeds rapidly. This also doubtless takes place slowly when dry, especially if the air contains any moisture. Eventually all the calcium hydrate is changed to calcium carbonate, the length of time required to effect the change varying from a few hours to a few weeks, according to the amount of excess of lime used in making the bordeaux mixture and the state of the weather. After this when wetted with rain the carbonate is gradually dissolved away, and finally all traces of the mixture disappear.

ADHESIVE PROPERTIES OF BORDEAUX MIXTURE.

One of the conditions necessary to the success of a preventive mixture like the bordeaux is that it must adhere well, so that neither wind nor rain can easily remove it.[1] When properly prepared, bordeaux mixture has adhesive properties that are truly remarkable, though according to Girard[2] several other copper mixtures adhere still better.

Before the evaporation to dryness of the drops of the mixture a number of factors come into play that have an effect on the sticking qualities of the spray. In the first place, the tendency of the liquid to collect in drops and fall from the leaf may prevent the surface from being evenly wetted, and also prevent any considerable amount from drying on the leaves. As far as can be judged from our present

[1]The addition of soap to bordeaux mixture to increase the adhesive or wetting capacity was first made in this country by Mr. B. T. Galloway, when trying to find some spray capable of wetting the very waxy young leaves of various cereals (see experiments in the treatment of rust affecting wheat and other cereals, Jour. of Mycology, Vol. VII, No. 3, 1893, p. 202). The addition of a considerable amount of soap greatly increases the tendency of the liquid to spread, instead of contracting to form large drops. A relatively enormous quantity (as much or even more than the amount of copper sulphate used) has to be added to secure this result. The chemical changes involved are almost entirely unknown. It is probable, however, that the whole of the calcium sulphate must be thrown down as an insolvent lime soap before the soap can yield a lather with the water. As the calcium sulphate is but slightly soluble, it is possible that even after a lather has formed from the sudden addition of a large amount of soap, it may continue to dissolve and precipitate the soap in solution and eventually cause the improved wetting effect due to the soap to disappear.

[2]*Girard, Aimé.* Recherches sur l'adhérence aux feuilles des plantes, et notamment aux feuilles de la pomme de terre, des composés cuivriques destinés à combattre leurs maladies (Compt. Rend., 114, No. 5, 1892, pp. 234–236).

knowledge, the principal copper salt present in bordeaux mixture is copper hydroxide $Cu(OH)_2$. As already mentioned, this salt is said to be entirely insoluble in the presence of calcium hydroxide in solution. After the neutralization of the calcium hydroxide, the rain, containing CO_2, ammonia, and nitrates, can act on the copper and effect the solution of small amounts. In 1887 Millardet and Gayon[1] published the record of an experiment bearing on the solubility of copper salts in bordeaux mixture. This is such an interesting article that we quote from it the following:

"On April 10 the following types of the mixture were prepared:

	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.
Copper sulphate.....grams..	16	15	15	15	15
Pure lime.....do....	3.36	3.36	6.72	13.44	26.88
Distilled water.....liter..	1	1	1	1	1

"As may be seen, in No. 1 there is 1 gram of copper sulphate in excess. No. 2 contains exactly the quantity of lime necessary to precipitate the copper sulphate. Tests made immediately after its preparation showed that there was no excess either of lime or of copper sulphate. No. 3 contains twice as much lime as No. 2, while No. 4 contains twice as much as No. 3. Finally No. 5, which is the formula in common use, contains twice the quantity in No. 4. These five mixtures were dried at once in a desiccating oven at 36° C.; then the products were pulverized in a mortar. Ten grams of each of the mixtures were spread in a thin layer between two Berzelius papers, supported below by a fine silk gauze, and were laid thus over five glass dializing jars having equal-sized mouths. The necessary precautions were taken to prove whether the paper and the gauze were capable of absorbing any considerable amounts of copper.

"On the evening of April 12 the five jars were exposed in a garden in an open place. After April 13 they were watered simultaneously by all the rains that fell. When it did not rain for some little time, each jar was given an equal quantity of rain water, which had been collected in advance. Each dializer was placed separately in a funnel draining into a test tube in which any water poured on the apparatus finally collected. Every two or three days the water from each of the five apparatus was removed simultaneously and analyzed separately. The following shows the order of appearance of the copper in the water which had drained through the mixture:

Mixture No. 1.....April 17
Mixture No. 2.....April 19
Mixture No. 3.....April 24
Mixture No. 4.....April 25
Mixture No. 5.....April 30

[1] *Millardet et Gayon.* Les divers procédés de traitement du mildiou par les composés cuivreux (Jour. d'Agr. prat., 51e ann., T. I, No. 2, May 19, 1887, pp. 701, 702).

"Other tests of the same nature, made by washing with rain water leaves of spindle wood and boxwood (Buxus) previously sprayed with these same five types of bordeaux mixture, gave analogous results. In general, the copper appeared in the water which had washed the leaves more quickly when the mixture contained less lime."

In 1890 the same authors[1] reported another experiment showing that bordeaux mixture, with 1 kilogram of copper sulphate and 300 grams of lime to 1 hectoliter of water, after being evaporated at 39° C. and left for days, gave no trace of soluble copper when washed in distilled water. They also state that it is the carbonating of the lime which, when completed, allows the copper to dissolve. This neutralization proceeds very slowly in dry air, and is greatly favored by a gentle rain of short duration. Doubtless showers, dew, fog, and even rather damp air favor the transformation.[2] The solvent action of rain water after all traces of lime in the mixture have been neutralized may probably be explained by the following hypotheses: The ammonia or nitrates contained in rain water dissolve a small amount of copper; the carbonic acid in solution probably dissolves some copper with chemical change; pure water, free from calcium hydroxide, dissolves slight amounts of copper hydroxide.

Regarding the first hypothesis, it is interesting to note that rain water often contains quite appreciable amounts of nitrogen, especially in the form of ammonium carbonate, ammonium nitrate, and ammonium nitrite. The results of an investigation by Failyer and Willard[3] on the rain falling at Manhattan, Kans., from 1886 to 1890 are of interest in this connection. From the tabulation of results by single rains for 1888–89 it can be readily seen that the amount of nitrogen per liter is greater in case of light showers than for heavy rains. In rain falling in showers of about 2.5 millimeters or less the amount of nitrogen in the form of ammonia was frequently 2 to 7 milligrams per liter, and in the form of nitric acid 3 to 6 milligrams per liter. On one occasion there were 12.227 milligrams of nitrogen per liter (10.376 milligrams as ammonia and 1.851 milligrams as nitric acid). The results for the four years from March, 1886, to March, 1890, give the mean total nitrogen as 0.522 milligram per liter, of which 0.388 was in the form of ammonia and 0.156 in the form of nitrates.[4] Petermann and Graftiau[5] found from an investi-

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[1] *Millardet et Gayon.* La bouillie bordelaise céleste (Jour. d'Agr. prat., 54e ann., T. I, No. 8, February 20, 1890, pp. 271, 272).

[2] The necessity for any excess of lime to be neutralized before the copper can be dissolved is a strong argument against using any greater amount of lime than is absolutely necessary.

[3] *Failyer, G. H., and Willard, J. T.* Ammonia and nitric acid in atmospheric waters (Kans. Expt. Sta. Second Annual Rept., 1889, pp. 128–132).

[4] There is probably some error here, as the sum of nitric acid and ammoniacal nitrogen is 0.544 milligram, not 0.522.

[5] *Petermann, A., and Graftiau, J.* Recherches sur la composition de l'atmosphère (Bull. Stat. Agr. Gembloux, No. 52, July, 1893, pp. 1–26; reviewed in Chem. Centralbl., 44, 1893, Bd. I, No. 15, pp. 710–712, and also quoted in A. de Ceris, Cronique agricole; Jour. d'Agr. prat., 57e ann., T. II, No. 36, Sept. 7, 1893, p. 322).

gation of all precipitation from January, 1889, to December 31, 1891, that at Gembloux, Belgium, the average amount of nitrogen per liter was 1.49 milligrams, of which 1.14 milligrams was ammoniacal and 0.35 nitric nitrogen. This makes the percentage ratio between ammoniacal and nitric and nitrous nitrogen 76 to 24, while at Rothamsted it is 75 to 25, at Montsouris 73 to 27, and at the German and the Italian stations 73 to 27. "The weight of nitrogen in the form of ammonium carbonate exceeds, therefore, that of the nitrogen in the form of ammonium nitrate or nitrite." The content of meteoric water in combined nitrogen was found by Petermann and Graftiau to be very variable, but nevertheless one fact of general interest appears to have been brought out, viz, a decrease of nitrogen from April to June or July, when the minimum is reached, and then an increase to a maximum in February. The amount of precipitation followed an exactly inverse rule. It is particularly interesting to note that fog, snow, and frost are characterized by a very large nitrogen content. In three cases the amounts of nitrogen in fog per liter were 2.60, 5.64, and 5 milligrams. As to the nitrogen content of dew, little seems to be known. From the high content of fog, however, it may be suspected that dew also is rich in combined nitrogen. These very small amounts of carbonate, nitrate, and nitrite are doubtless of great importance in effecting the solutions of small amounts of the nearly insoluble copper salts present not only in bordeaux mixture, but in many other fungicides. Probably cuproammonium compounds are formed which are soluble in water. As to the details of the process of solution, however, little is known.

It is quite probable that the carbonic acid dissolved in atmospheric water may dissolve small amounts of the cupric hydroxide. However, the amount of CO_2 present, even in a saturated solution, is very small. Puchner[1] found the amount of CO_2 in the open air in the vicinity of Munich, Germany, to vary constantly and considerably at all heights observed (1 centimeter, 2 meters, and 10 meters above the ground). The following table illustrates the great extent of the variations, the amounts being the cubic centimeters of CO_2 contained in 10 liters of air, all observations being reduced to 0° C. and 760 millimeters pressure:

Amount of carbonic acid in the air in an open field near Munich, Germany.

Height.		Extremes.	Means.	Variation of minimal content.
				Per cent.
1 centimeter above ground	Day	1.998–6.805	4.817	242.30
	Night	1.750–4.318	2.568	146.74
	Mean	1.869–5.561	3.692	194.52
2 meters above ground	Day	1.942–7.063	5.121	263.69
	Night	1.750–5.571	3.821	218.84
	Mean	1.583–6.317	4.471	241.01
10 meters above ground	Day	1.583–4.481	2.898	185.59
	Night	2.005–4.888	2.893	144.28
	Mean	1.794–4.689	2.896	164.93

[1] *Puchner, H.* Untersuchungen über den Kohlensäuregehalt der Artmosphäre (Forsch. Geb. Agr. Physik, 15, 1892, pp. 296–383; reviewed in Biedermann's Centralbl. Agr. Chem., 23, Heft 7, 1893, pp. 433–440. Original not seen).

It was observed that wind diminished and snow increased the proportion of the CO_2 in the air. Puchner made many observations on air from very different situations and under as diverse weather conditions as possible. In the great majority of determinations (1,527) the content varied between 2 and 5.5 volumes in 10,000 volumes of air, and averaged 3.67 to 10,000.

According to Bunsen and Pauli,[1] 1 volume of water under 760 millimeters pressure dissolves:

At—	Parts by volume of CO_2.
0° C.	1.7967
5° C.	1.4497
10° C.	1.1847
15° C.	1.0020
20° C.	0.9014

According to Regnault,[2] the specific gravity of CO_2 compared with air is as 1 to 1.5341. If there were 3.67 parts by volume of CO_2 to 10,000 of air, the average as given by Puchner, the pressure would be of course 0.000367 atmosphere, and 1 liter of water saturated with CO_2 would contain:[3]

At—		Milligrams CO_2.
Degrees C.	Degrees F.	
5	41	0.94
15	59	0.62
20	68.2	0.55

The extreme variations in content of the air in CO_2 as given by Puchner would have caused the amount to be halved at minimum content or doubled at maximum. The amount present in rain or dew may sometimes be even less than would be indicated by the figures given above, since the water might not be fully charged with CO_2 in all cases. Gayon[4] found that 1 liter of water charged with CO_2, at ordinary pressure, was able to dissolve 40 milligrams of cupric hydroxide at a temperature of 15° C. If fully saturated, this amount of water at 15° C. and 760 millimeters pressure would contain 1.002 volumes of CO_2, weighing 1.8722 grams. This would indicate that the carbonic acid contained in water can dissolve about 2.15 per cent of its own weight of cupric hydroxide. It seems scarcely possible that the minute quantities of CO_2 contained in rain water or dew alone could dissolve enough copper

[1] Quoted from Roscoe and Schorlemmer, Ausführliches Lehrb. der Chem., Bd. I, Braunschweig, 1877, p. 536.

[2] Quoted from Roscoe and Schorlemmer, l. c., p. 527.

[3] Calculated from Fowne's Manual of Chemistry (New American edition, from twelfth English edition; Lea Bros. & Co., Philadelphia, 1885, p. 1032), using Table VII for the weight of air at the temperature given, and 760 millimeters pressure.

[4] *Gayon et Millardet.* Traitement du mildiou par le mélange de sulfate de cuivre et de chaux (Jour. d'Agr. prat., 49e ann., T. II, No. 46. Nov. 12, 1885, p. 710). Gayon found that pure water dissolved slight traces of cupric hydroxide at 15° C.

to poison any but the most delicate plants. Probably, as claimed by Gayon, the copper hydroxide present is slightly soluble in pure water. Nägeli[1] found, as will be seen later, that the purest of distilled water would absorb enough copper from carefully cleaned copper or gold coins (one-tenth copper) to make the water highly poisonous to Spirogyra after a few hours. It is possible that the drops of rain or dew standing for some time on the leaves or other parts of plants may absorb from superficial cells sugar or other substances that may serve to increase the solubility of the copper. Some color is given to this hypothesis by the observation of Büsgen,[2] who suspended carefully cleaned grapes in water so that the pedicel and point of attachment were not wetted, and after twelve hours detected sugar in the water. He also noted in his study of germination of parasitic fungi that the germ tubes of many species are attracted to the boundaries between two epidermal cells where they penetrate. If leaves are placed in water full of bacteria the latter collect at the boundary line between the cells, proving, in Büsgen's opinion, that there is a faster exomosis of dissolved matters here than over the lumen of the cells.

The germ cells of many parasitic fungi are strongly attracted to the guard cells, as are also bacteria. Büsgen succeeded in obtaining a thick black precipitate on the epidermis of leaves of *Eranthemum pulchellum* (Acanth.) and *Barberea vulgaris* (Crucif.) that had been wetted with a few drops of ammoniacal solution of silver nitrate. Usually the epidermal cells remained entirely free from the precipitate. Only once, with *Eranthemum pulchellum*, was a heavier precipitate obtained, and this was along the boundaries of the epidermal cells. The wall was found to have no power to precipitate the silver solution, so the author concluded that the precipitate was caused by some matter which had diffused into the ammoniacal silver nitrate from the interior of the cells. It may be that the guard cells, lacking, as they do, intracellular connections with the neighboring cells,[3] may more often contain a great excess of the products of assimilation. Now, according to Brown and Morris,[4] the first product of assimilation is cane sugar, and only when it accumulates in excess is starch formed. Moreover, the maltose formed from the starch under the influence of diastase may be like cane sugar and sirup in its action on copper salts. Fresenius[5] states that in the presence of sugar or similar organic substances caustic alkalies produced

[1] *Nägeli, C.* Ueber oligodynamische Erscheinungen in lebenden Zellen. Separatabdruck aus den Denkschriften der schweizerischen naturforsch. Ges., 33, Heft I, 1893.

[2] *Büsgen.* Ueber einige Eigenschaften der Keimlinge parasitischer Pilze (Bot. Zeit., 51, Abth. I, Heft 3 and 4, 1893, p. 67).

[3] *Kienitz-Gerloff, F.* Die Protoplasmaverbindungen zwischen benachbarten Gewebselementen in der Pflanze (Bot. Zeit., 49, 1891, No. 4, col. 58).

[4] *Brown, H. T., and Morris, G. H.* A contribution to the chemistry and physiology of foliage leaves (Jour. Chem. Soc., England, 1893, pp. 604–683).

[5] *Fresenius, C. R.* Manual of Quantitive Chemical Analysis, translated and newly revised by S. W. Johnson, twelfth edition. John Wiley & Sons, New York, 1893, p. 176.

precipitates which are soluble in excess of the precipitants. Sugar and sirup have been used extensively in France to dissolve in part the cupric hydroxide and basic carbonate occurring in fungicides.

It would take but very little sugar to have as much solvent power as the traces of ammonia and CO_2 in rain or dew. Of course it is possible that other substances besides sugar might find their way from the plant cell to the drops of liquid containing the copper, and aid in dissolving it. Pons[1] found that although neutral and acid salts of copper are strongly insoluble in fats and fatty matters, the hydrate and basic salts are rather easily soluble. He believes the fatty matters of the cuticle of plants are comparable with animal fats and not with wax, and consequently that they would be likely to exercise a solvent action on copper salts.

Finally, it is possible that substances secreted or excreted by cells of parasitic fungi may effect the solution of a small amount of copper. The ferments and cements secreted by the hypha tips of many parasitic fungi, especially when they come into contact with a solid body and in case the solid particle contains copper, might dissolve a considerable amount just about the delicate growing tip.

If, as is quite possible, the copper is quickly absorbed by the fungous cells, the solvent could then take up another portion, which in turn would be absorbed by the fungus, and so on. In this manner poisoning might ensue, even though the copper were only very slightly soluble. From what has been said it can be seen that further researches on the conditions governing the solubility of the copper compounds used as fungicides are very much needed. Scarcely anything has been definitely settled. Almost all writers have overlooked a number of the conditions influencing solubility.

AMOUNTS OF COPPER IN SOLUTION NECESSARY TO KILL FUNGI AND ALGÆ.

This question has a very direct bearing in connection with any hypothesis to account for the efficiency of bordeaux mixture or any other fungicide containing copper.

To Benedict Prevost belongs the credit of not only first discovering the fungicidal properties of copper and its compounds and applying them in practice, but also of determining with some degree of accuracy the amount of copper necessary to kill spores or prevent their germination. In a work published in 1807[2] he gives an account of the discovery of the effect of the copper on spores of wheat smut (*Tilletia tritici*). This account is so interesting that a translation is here given:

"Having found that a great number of substances when added to

[1] *Pons, B.* Le cuivre et le mildiou (Jour. d'Agr. prat., 54e ann., T. II, No. 34, 1890, p. 276).

[2] *Prevost.* Mémoire sur la cause immédiate de la carie ou charbon des blés et de plusieurs autres maladies de plantes et sur les préservatifs de la carie (Montauban, 1807, 4, p. 85).

water give it the property of destroying the germs of the seeds of the smut, it remained for me to investigate which of these substances had in the highest degree all the qualities of a preventive applicable to agriculture. I had commenced to do this systematically when a fortunate accident greatly shortened this labor. Having washed some smut first with well water several times, afterwards with water which had been distilled in a large copper alembic, and having left it some time in a glass goblet with this water, I placed in a mounting glass half full of very pure distilled water some drops of water from the goblet, containing many hundred germs of seeds of smut, which, to my great astonishment, did not germinate or germinated very poorly, while other cultures, made under similar circumstances, except for the copper, germinated well. I decided, therefore, to direct my investigation first and principally to copper and the copper salts."

He afterwards found that 10 or 12 milligrams of copper, in a fine powder, in 150 cubic centimeters of water, prevented the germination of *Tilletia tritici*, which fungus was used in all his experiments to determine the effect of copper. Plates of cleaned or uncleaned brass or copper, copper coins, and bell metal produced the same effect.[1]

His experiments made with copper sulphate dissolved in ordinary well water[2] showed that at a temperature of $6\frac{1}{4}^{\circ}$ to $7\frac{1}{2}^{\circ}$ C. the spores failed to germinate in a solution containing 1 part in 250,000;[3] 1 part in 600,000, or even 1 in 1,000,000, sensibly retarded germination. Soaking one-half hour in a solution of 1 to 10,000 deprived spores of their power of germination, although they were washed immediately after removal from the copper sulphate.[4] In repeating the experiments at a higher temperature he found that the Tilletia sometimes germinated in water containing 1 part to 200,000, and that soaking some hours in a solution of 1 to 10,000, followed by washing in water, did not prevent the spores from germinating.[5] He found, furthermore, that the well water used in making the solution caused the formation of a white, bluish, or greenish precipitate, which he supposed might be calcium sulphate and copper carbonate. If the solution was filtered the filtrate had very much less effect on the spores. He suggests that the precipitate might act on the smut or that the filter might act on the copper sulphate left in the solution.[6]

Experiments were made with distilled water also. At a high temperature 1 part to 200,000 had scarcely any effect, but 1 to 100,000 pre-

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[1] *Prevost, l. c.*, pp. 57, 58, § 139.

[2] *Prevost, l. c.* In a footnote on page 61, he states that this well water was very clear, good for drinking purposes, and did not contain more than one twenty-eight hundredth of its weight of foreign matter in solution, this being principally calcium salts and sodium chloride.

[3] *Prevost, l. c.*, p. 59, § 145. This was the ordinary salt, $CuSO_4.5H_2O$, making, as he calculates, 1 part in 400,000 of the water-free sulphate.

[4] *Prevost, l. c.*, p. 59, § 44.

[5] *Prevost, l. c.*, p. 60, § 45.

[6] *Prevost, l. c.*, p, 61, §§ 152, 153.

vented all germination. Soaking the spores several hours in a solution containing 1 part to 8,000 and washing afterwards scarcely injured them. A half hour's immersion in a solution of 1 to 2,400 at 13¾° C., however, destroyed all power of growth.[1] Often the weaker solutions prevented all germination except on the surface of the culture, the spores wholly immersed in the liquid uniformly failing to grow.

A solution made by treating copper with vinegar and diluting till it contained 1 part copper in 30,000 (about 1 in 10,000 of copper acetate) destroyed the germinative power of spores soaked in it four hours at 22½° to 25° C.,[2] but if the smut is soaked only two hours, or if there is only 1 part of copper to 75,000 (1 to 25,000 of acetate) in solution, germination is only retarded.

The next important researches on this subject were those of Millardet in collaboration with Gayon, published in 1885.[3] These authors found that a solution of 2 or 3 parts copper sulphate to 10,000,000 of water was capable of killing the zoospores of the downy mildew of the grape (*Plasmopara viticola*) in a short time, while a solution of 1 part lime to 10,000 of water was necessary to accomplish the same result. An examination of the water of Millardet's well, which was 11 meters deep and furnished with an old copper pump, showed a copper content of 5 milligrams per liter, or more than ten times as much as is required to kill the zoospores of *Plasmopara viticola*. This explained the fact previously observed by Millardet, that the spores of grape mildew refused to germinate in this water.

In a subsequent paper[4] the author published the record of experiments with water taken September 23, after a heavy rain, from the tips of grape leaves that had been sprayed twice with bordeaux mixture, on June 15 and July 10, and still showed evident traces of the mixture. The water so collected prevented the germination of Plasmopara spores placed in it. The water showed no alkaline reaction and hence did not contain any appreciable quantity of lime. Its action must, therefore, have been due to the copper it contained. Unfortunately the authors made no analysis and consequently did not know the amount of copper actually in solution in this water.

In 1889 Dufour[5] published an account of some trials made to deter-

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[1] *Prevost, l. c.*, p. 61, § 154.

[2] *Prevost, l. c.*, p. 66, § 165.

[3] *Millardet.* Traitement du mildiou par le mélange de sulfate de cuivre et de chaux (Jour. d'Agr. prat., 49e ann., T. II, No. 46, 1885, pp. 707–710; translated in F. Lamson-Scribner's report on the fungous diseases of the grapevine, Section of Vegetable Pathology, Division of Botany, Bull. No. 2, U. S. Dept. of Agr., 1886, Appendix C, pp. 108–112).

[4] *Millardet et Gayon.* Recherches nouvelles sur l'action que les préparations cuivreuses exercent sur le Peronospora de la vigne (Jour. d'Agr. prat., 51e ann., T. I, No. 4, 1887, p. 124).

[5] *Dufour, J.* Note sur l'action du sulphate de cuivre sur la germination de quelques champignons (Landw. Jahrb. der Schweiz, Bern, 1889, p. 97; cited from Wüthrich, Zeitsch. f. Pflanzenkrank., Band II, Heft 1, 1892, p. 17. Original not seen).

mine what strength of copper sulphate solution was necessary to prevent the germination of various fungi (*Claviceps purpurea*, *Fusicladium pyrinum*, Pleospora, and Phragmidium). All germinated in a solution of 1 to 1,000,000. Germination was commonly abnormal in solutions of 1 to 100,000.

Wüthrich[1] in 1892 gave an account of an extensive series of experiments to determine the influence of various metallic salts and acids on the germinative power of the spores of several fungi. Copper sulphate was one of the salts tested. The following brief summary will show the results of the experiments where copper sulphate was used:

(1) Conidia of *Phytophthora infestans* in water—
1 part to 800,000[2] (0.00001 equiv.[3]); normal germination.
1 part to 80,000 (0.0001 equiv.); no zoospores; some direct germination.
1 part to 8,000 (0.001 equiv.); all germination prevented.
(2) Conidia of *Phytophthora infestans* in malt extract solution—[4]
1 to 800,000 (0.00001 equiv.); germination as in pure malt extract solution.
1 to 80,000 (0.0001 equiv.); germination noticeably hindered.
1 to 8,000 (0.001 equiv.); no germination.
(3) Free zoospores of *Phytophthora infestans*—
1 to 800,000 (0.00001 equiv.); normal movement and germination.
1 to 80,000 (0.0001 equiv.); motion stopped in one minute; after fifteen hours very few germinated.[5]
1 to 8,000 (0.001 equiv.); motion stopped instantly; no germinations.
(4) Conidia of *Plasmopara viticola*—
1 to 800,000 (0.00001 equiv.); germination as in pure water.
1 to 80,000 (0.0001 equiv.); no germination.
(5) Free zoospores of *Plasmopara viticola*—
1 to 800,000 (0.00001 equiv.); normal movement and germination.
1 to 80,000 (0.0001 equiv.); motion stopped in one minute; no germination after fifteen hours.
(6) Spores of *Ustilago avenæ*—
1 to 800,000 (0.00001 equiv.); many germinations, but plainly fewer than in pure water.
1 to 80,000 (0.0001 equiv.); germination greatly hindered; only a few short germ tubes.
1 to 8,000 (0.001 equiv.); no germination.

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[1] *Wüthrich.* Ueber die Einwirkung von Metallsalzen und Säuren auf die Keimfähigkeit der Sporen einiger der verbreitesten parasitischen Pilze unserer Kulturpflanzen. Zeitsch. für Pflanzenkrank., Band II, Heft 1 and 2, 1892, pp. 16–31 and 81–94, Tabellen 1–31.

[2] Of $CuSO_4.5H_2O$ by weight.

[3] The solutions were prepared according to the chemical equivalents of the substances. The number given indicates the amount of hydrogen by weight which 1 part by weight of the solution can replace or combine with. Thus 1 part of $CuSO_4.5H_2O$ to 800,000 of water is 1 part of Cu to about 200,000 of water, but copper being bivalent here the solution would have the same chemical equivalence as one of H to 100,000 water.

[4] Malt extract was used isotonic with a potassium nitrate solution of 0.15 equiv. (about 1 part to 75 of water by weight). By isotonic is meant having the same osmotic action as determined plasmolysis of epidermal cells of *Tradescantia zebrina*.

[5] Zoospores which had recently issued from the conidia when placed in the solution did not germinate, while a few of those nearly through swimming about pushed short germ tubes.

(7) Spores of *Ustilago avenæ* in malt extract solution—
1 to 8,000 (0.001 equiv.); all germinated; countless conidia formed by budding.
1 to 800 (0.01 equiv.); many promycelia; few conidia.
1 to 160 (0.05 equiv.); only scattered germination; no conidia.
1 to 80 (0.1 equiv.); no germination.
(8) Uredospores of *Puccinia graminis*—
1 to 800,000 (0.00001 equiv.); normal germination.
1 to 80,000 (0.0001 equiv.); scarcely different from pure water.
1 to 8,000 (0.001 equiv.); only scattering germinations; germ tubes short; spores mostly discolored.
1 to 800 (0.01 equiv.); no germination.
(9) Æcidiospores of *Puccinia graminis*—
1 to 800,000 (0.00001 equiv.); normal germination.
1 to 80,000 (0.0001 equiv.); germination plainly hindered.
1 to 8,000 (0.001 equiv.); no germination; spores discolored.
(10) Conidia of *Claviceps purpurea*—
1 to 80,000 (0.0001 equiv.); no germination.
1 to 8,000 (0.001 equiv.); no germination.[1]

In 1893 Carleton[2] published the results of a considerable number of experiments on the germination of the uredospores of *Puccinia rubigo-vera* and *P. graminis* in solutions of various substances in distilled water. Among them a number of trials were made with copper salts. The results obtained were quite variable, but usually 1 part in 1,000 of copper sulphate or nitrate prevented almost all germination. With copper acetate 1 to 10,000 prevented most of the germination of *Puccinia graminis*, while 1 to 1,000 of copper chloride prevented all germination.

As may be seen from the results summarized above, there is considerable difference in the susceptibility of different kinds of fungi. For instance, in case of *Plasmopara viticola*, 1 part of $CuSO_4.5H_2O$ to 80,000 H_2O was fatal, while in the case of *Ustilago avenæ* in a nutrient solution 1 part to 160 did not prevent all germination.[3] Some of the mold fungi are known to be remarkably indifferent to copper solution. Pfeffer[4] notes that *Penicillium glaucum* can grow in a somewhat

[1] It is of interest to note that mercuric chloride solution was about equal (by equivalents) to copper sulphate against *Plasmopara viticola* (analogous to 4 and 5), from 10 to 20 times as effective against *Ustilago avenæ* in malt extract solution (7), and about 10 times as effective in all other cases (0.001 (equiv. $HgCl_2$) equals about 1 to 7,400 by weight). Zinc and iron sulphates were much less effective than copper sulphate. From the results of these experiments it would seem that mercuric salts are well worthy of further trial as fungicides.

[2] *Carleton, M. A.* Studies in the biology of the Uredineæ I. Notes on germination (Bot. Gaz., 18, No. 12, 1893, pp. 447–457; see also *Hitchcock* and *Carleton*, preliminary report on rust of grain, Kans. Exp. Sta. Bull. No. 38, 1893, pp. 7–10).

[3] It is interesting to note that the remarkable resistance of *Ustilago avenæ* to copper is also shown by the fact that the treatment of seed oats with copper sulphate solution fails to prevent the smut entirely, although perfectly effective for *Tilletia tritici* and *T. fœtens* on wheat (see *Kellerman* and *Swingle*, Additional experiments and observations on oat smut, made in 1890, Kans. Exp. Sta. Bull. No. 15, 1891).

[4] *Pfeffer.* Pflanzenphysiologie, 2, p. 454.

concentrated solution of copper sulphate. According to Manasein,[1] solutions of this salt must be as strong as one-fourth of 1 per cent before there is any appreciable effect on the growth of this fungus. Winckiewicz[2] found Mucor to be unaffected by a one-fifteenth of 1 per cent copper sulphate in a Buchholtz-Pasteur nutrient solution at 20° to 30° C. Nägeli, in a posthumous work published in 1893,[3] gives an exceedingly interesting account of some astonishing effects of minute amounts of copper on certain species of Spirogyra. Although none of his experiments were on fungi, the results are of the greatest value, not only in throwing light on the manner in which copper acts on living cells, but also in pointing out possible sources of error in experimenting on this subject. In the light of his work it is probable that none of the previously published results are entirely trustworthy; they certainly should not be accepted as such till more is known of the conditions under which they were obtained. Nägeli worked with a Spirogyra having cylindric nuclei, and found the death of the cells to result in several very different ways, according to the strength of the toxic agent[4] employed, as follows:

(1) Very strong solutions cause physical injury, extreme plasmolysis, etc.

(2) Weaker solutions cause what he calls chemical poisonous action. In this strength the plasma contracts and the turgescence of the cell is lost. The chlorophyll bands, without changing their position in the protoplasm, become more cylindric and sometimes break up.

(3) Weaker solutions cause the oligodynamic action discovered by Nägeli. The action on the chlorophyll bands is very characteristic. They loosen from the protoplasmic membrane and draw into the interior of the cell without changing their form in cross section. Usually they straighten somewhat and may become entirely straight. Finally the chromatophores contract to a solid lump, surrounding the rounded nucleus. At first the protoplasm is not changed, but finally it becomes dark and retracts from the wall and the cell becomes flaccid.[5]

(4) Still weaker solutions cause changes resembling those of "natural death," where the chlorophyll bands retain their position on the parietal

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[1] *Manasein*, noted by Loew, Dr. O. Ein natürliches System der Giftwirkungen (München, 1893, p. 36).

[2] *Winckiewicz, Bronislaw.* Das Verhalten des Schimmelgenus Mucor zu Antiseptica und einiger verwandten Stoffen mit besonderer Berücksichtigung seines Verhaltens zu zukerhaltigen Flüssigkeiten (Inaugural-Dissertation, Dorpat, 1880; reviewed in Bot. Centralbl. I, Jahrg. 4, No. 46, 1880, pp. 1411–1413). The nutrient solution was 10 grams candy sugar, 1 gram ammonium nitrate, and ½ gram potassium phosphate to 100 cubic centimeters of water.

[3] *Nägeli.* Ueber oligodynamische Erscheinungen in lebenden Zellen, mit einem Vorwort von S. Schwendener und einem Nachtrag von C. Cramer (Separatabdruck aus den Denkschriften der schweizerischen naturforsch. Ges. 33, Heft I, 1893, pp. 1–51; Kommiss.-Verlag v. H. Georg, Basel).

[4] *Nägeli, l. c.*, p. 33.

[5] *Nägeli, l. c.*, pp. 36–38.

layers of protoplasm, but become more nearly parallel to the axis of the cell; lose their regular arrangement, and then fall into small pieces; their cross section becomes oval or roundish; in the protoplasm the streaming motion ceases; in the cell fluid small granules are formed, which finally often fill the whole cell cavity; and the parietal layer becomes darker, appears granular, and withdraws from the wall, the cell then losing its turgescence.[1] Nägeli is inclined to consider this last-named action as a very much reduced chemically poisonous effect, possibly caused in nature by accumulation of excretions of the plant itself. He holds the oligodynamic effect to be entirely different, especially from chemical poisoning.[2]

Most poisonous solutions could produce all three kinds of poisoning, but some, such as metallic copper, did not produce chemical poisoning, probably because too sparingly soluble. Ammonium nitrate, on the other hand, produced no oligodynamic action at any concentration, although 1 to 1,000 or 1 to 10,000 caused chemical poisoning.[3] Soluble copper salts were found to cause death in all four ways. Copper, silver, and mercury, in about the order named, were found to be exceedingly poisonous. When placed in contact with pure distilled water in the pure metallic state, although but very slightly soluble, they rapidly rendered the water capable of producing strong oligodynamic effects. Gold and platinum, on the other hand, which he found insoluble in pure water, produced no poisonous effect. One part of metallic copper in 77,000,000 parts[4] of water was found to cause strong oligodynamic poisoning in a few minutes, and Nägeli thinks 1 to 1,000,000,000 capable of causing plain oligodynamic action and of killing Spirogyra.[5]

A very important fact brought out during the course of the investigation was that if metallic copper, mercury, difficultly soluble copper, or silver compounds were left in pure water in glass vessels the glass after a time acquired the same properties, and upon removal of the poisonous substance and oligodynamically active water the vessels would speedily render poisonous a fresh quantity of pure water placed in them. Rather strong solutions of the readily soluble copper, silver, and mercury salts produce the same after effect. On the other hand, large surfaces often neutralized the poisonous action of water previously rendered oligodynamic by absorbing the metals or difficultly soluble salts. Various insoluble substances, especially in a state of fine subdivision, possessed the same power; among these were roll sulphur, flowers of sulphur, graphite, coke, anthracite, iron superoxide, starch, cellulose (Swedish filter paper, cotton, linen, or wood), silk, wool, stearic acid paraffin, and also Spirogyra cells themselves when added in considerable numbers. What Nägeli calls micellar soluble substances, such as dextrin, albumen, glue, etc., also neutralize the poisonous action

[1] *Nägeli, l. c.*, pp. 32, 40, 42, 43.
[2] *Nägeli, l. c.*, p. 45.
[3] *Nägeli, l. c.*, p. 42.
[4] *Nägeli, l. c.*, p. 23.
[5] *Nägeli, l. c.*, pp. 24, 25.

of oligodynamic water. It is an interesting fact that chemically related molecular soluble substances, such as sugar, possess no such power. Dilute hydrochloric and nitric acids were found to be able to destroy the after effects of the glass vessels, as did also prolonged boiling in large quantities of water.

Nägeli gives the following explanation of the after effect of glass vessels which had previously contained water rendered oligodynamic by metals:

"If a piece of copper is placed in pure water containing a little oxygen and carbonic acid, there occurs a slow but constant separation of particles of copper, which distribute themselves through the water. As the solution becomes more concentrated the number of insoluble copper particles clinging to the walls must increase. After the solution reaches the saturation point, a process of solution can still continue, inasmuch as the number of particles that attach themselves to the walls is greater than the number that loosen from the walls and enter the liquid. Finally a condition of equilibrium is reached when the copper coating of the walls loses as many molecules as it receives. If one pours such a copper solution into another (clean) glass vessel, the concentration decreases until a new equilibrium is established. If one puts pure water into a glass having a coating of copper, particles of copper separate from the walls and enter the water until a ratio between solution and precipitation is reached corresponding to the quantity of copper. There is a definite ratio between the concentration of the copper solution and the thickness of the copper coating of the wall. The quantity of copper adhering to the walls in relation to the amount in solution is greater the larger the surface of the walls is in relation to the amount of water."

It is therefore easy to see how adding insoluble matters, such as sulphur, graphite, cotton, etc., or simply using large glass surfaces, can destroy the oligodynamic properties of water. Nägeli considers that the micellar soluble bodies, such as gum, dextrin, etc., may have copper deposited on the surface of the individual micellæ ("invisible small crystalline bodies") and thus present very extended surfaces for the precipitation of copper. The molecules of the molecular soluble substances, such as sugar, do not offer any surface to which the copper can attach itself, and hence these substances do not destroy the poisonous properties of oligodynamic water. Very likely adding a large quantity of Spirogyra cells increases the surface capable of receiving copper, not only because of the gross area of the cell wall, but also by means of various micellar soluble substances they contain and secrete. Dilute acids probably clean the walls of the vessels, showing after effects by forming readily soluble salts of copper, silver, or mercury, as the case may be, which are easily washed away.

Cramer carefully went over the work after Nägeli's death and in the main points confirmed his results.[1] He found perfectly neutral distilled

[1] *Cramer, C.* Schlussbemerkung, *Nägeli, l. c.*, pp. 44–51.

water to absorb carefully purified metallic copper or mercury, even when free oxygen or carbonic acid were excluded. After standing in contact with one of these metals four days, the water exhibited strong oligodynamic action on Spirogyra within five minutes. He found that a 1 to 1,000,000 copper sulphate solution produced oligodynamic action in thirty-five minutes, a 1 to 10,000,000 mercuric chloride solution caused unmistakable oligodynamic action within a day, and a 1 to 100,000,000 in the course of two or three days, but more or less doubtful. He was, however, unable to obtain any effect with solutions as dilute as 1 to 1,000,000,000.

The use of distilled water poisoned from contact with copper of glass vessels showing after effect, and the neutralization of the poisonous effect of very dilute solutions by adding too many spores, would vitiate the results obtained.

In the light of the results of Nägeli's investigations it is probable that many accidents have occurred, entirely unsuspected, in the course of previous tests of the effect of copper and other substances on the germination of fungi. It is to be hoped that in the future the valuable suggestions furnished by Nägeli's work may be made use of by experimenters in this field.

STAGE OF DEVELOPMENT OF FUNGI WHEN ACTED UPON BY THE COPPER IN FUNGICIDES.

A knowledge of the condition of the fungus at the time the poisonous action of copper takes place would throw much light on the exact action of bordeaux mixture. Some discussion of this matter is therefore given here.

(1) The spores may be prevented from germinating by what, for lack of a better word, may be called inhibitory action,[1] in which the spores, though kept from germinating, are not killed, or at any rate are not killed at once. It may be that the first steps toward germination do take place, and upon the protoplasm becoming active and the wall being thinned or broken preparatory to the pushing of the germ tubes, the poison is able to act so strongly that all further progress in germination is stopped or the protoplasm killed outright. Wüthrich[2] observed that when free swimming zoospores of *Phytophthora infestans* were placed in a copper sulphate solution of 0.0001 (equiv. 1 to 80,000), those

[1] Prevost found that solutions of copper salts which were very much stronger than those capable of preventing germination did not kill the spores of *Tilletia* if after a few hours the spores were washed in water. Thus, while a solution of 1 gram of copper sulphate to 100,000 of water prevented germination, soaking some hours in a solution of 1 to 8,000, followed by washing in water, scarcely affected the spores (l. c., p. 61). It may be that the weak solution, capable of preventing germination, exerted simply a restraining action and did not kill the spores at all. There is some evidence to show that the action of mercuric chloride on spores of bacteria is merely of a restraining character (Univ. of Mich. Bull., 1892).

[2] See p. 24.

which had recently quitted the conidia did not germinate, while a few of those nearly through swimming pushed short germ tubes. Such a case would hardly occur in nature unless the copper coating on the leaves was very unequal and rain or dew drops free from copper, in which zoospores had formed, afterwards became confluent with other drops containing copper.

(2) The protoplasmic content of the spore may be killed outright in a short time, before germination has even commenced. It is probable that in the majority of cases bordeaux mixture and other copperfungicides act on fungi before the spores germinate, either killing the spores outright or else preventing germination by inhibitory action.

(3) It is possible that the copper hydroxide may be scarcely, if at all, injurious to the germ tube, but through negative chemiotropic action may prevent the germ tubes from entering the plant. In this event the copper dried on the leaf or absorbed by the cuticle would be at the bottom of the drop, and if possessing negative chemiotropic powers would hinder or prevent the entrance of the germ tube. Of course if this action takes place the coating of copper salt over the leaf would have to be nearly continuous to prevent entrance. So far as known to the writer this hypothesis has not before been proposed to account for the action of fungicides. Reinhardt[1] in 1892 noticed that the mycelia of various species of Peziza are very sensitive to stimuli, being attracted in the culture medium in which they grow to any richer in nutritive substances. Other species of fungi and especially bacteria occurring in the same culture medium were found to cause a repulsion of the hyphæ. In some cases as soon as the Peziza hyphæ reached the boundaries of the other colonies, or even before the limits of a colony were reached, in the case of bacteria, the hyphæ were stopped in their growth. The very interesting researches of Miyoshi,[2] published in 1894, throw still more light on the sensitiveness of fungi to chemiotropic stimuli. He showed that the mycelial threads of Mucor, Penicillium, Phycomyces, etc., could be made to grow into living leaves of Tradescantia and other plants very much like parasitic fungi, if the leaves had been previously injected under pressure with a 2 per cent flesh extract. *Penicillium glaucum* and *Botrytis bassiana* penetrated far into the tissue of the leaves, while to noninjected leaves they were indifferent. Probably substances existing naturally in the host plant impel parasitic fungi to grow into the stomata or bore through the wall, as the case may be. Büsgen,[3] in 1893, found the hyphæ of *Botrytis cinerea* and other para-

[1] *Reinhardt.* Das Wachsthum der Pilzhyphen. Ein Beitrag zur Kenntniss des Flächenwachsthums vegetabilischer Zellmembranen (Pringsheim, Jahrb. f. Wiss. Bot., 23, Heft 4, 1892; reviewed in Bot. Centralbl., 51, 1892, 380–383).

[2] *Miyoshi, Manabu.* Ueber Chemitropismus der Pilze (Bot. Zeit., 52, Heft I, 1894, Abhandl., 1, p. 28, Pl. I).

[3] *Büsgen.* Ueber einige Eigenschaften der Keimlinge parasitischer Pilze (Bot. Zeit., 51, Abhandl., Heft 3 and 4, 1893, pp. 53–72, Pl. 3; see article by the writer on the chemical and physical structure of bordeaux mixture, Jour. of Mycology, Vol VII, 1895, p. 365–371.

sitic fungi to be attached to the host tissue by substances diffusing into the water containing the fungi. Miyoshi brought out the important point that repulsive action is exerted by many substances,[1] especially poisons, and that if present in sufficient quantity these repulsive matters were able to overcome the attraction exerted by others. Thus, a mixture of one-half of 1 per cent absolute alcohol with 1½ per cent flesh extract had a repulsive action on hyphæ of *Mucor stolonifera*. May it not be that the germ tubes of parasitic fungi germinating in a drop of rain or dew resting on a leaf with, say, bordeaux mixture, will be more strongly repelled by small amounts of copper entering solution at the leaf surface than they are attached by matters given off by the host plant?

(4) The germ tube may be so weakened by copper in solution as to be unable to overcome the natural barriers to its entrance interposed by the host plant. Here, again, the action would be practically continuous from the first appearance of the germ tube. Ward[2] has called attention to the struggle between host and parasite and has shown that many different factors come into play. May it not be that the effect of the copper, though insufficient to kill the fungus or even stop its growth, in many cases turns the balance in the struggle in favor of the host plant? In this event it is easy to see that spraying with copper mixture would be much more effective when conditions were favorable to the host and unfavorable to the parasite.

(5) The germination tubes may be prevented from growing or be killed only upon contact with solid particles of copper or its compounds or with the cuticle or other parts of the host impregnated with copper. The action in this case would not be continuous, but would usually be felt only where the germ tubes were of some length. To secure perfect protection, if the copper acts in this way, it would be necessary to have an almost continuous coating of copper over the surface of the leaf. Büsgen[3] has shown that the germ tubes of a number of parasitic fungi studied by him form peculiar swellings when they touch a hard body, and enter into most intimate contact with it. In *Botrytis cinerea* and other related fungi an unusual branching end of the hypha ensues and a "brush" or holdfast of considerable size is formed. A cementing substance is formed which serves to fasten such very firmly, even to glass. It is also possible that the ferments secreted by many such fungi are produced in special abundance here. Any secretion produced

[1] Calcium nitrate ($Ca(NO_3)_2$), magnesium sulphate ($MgSO_4.7H_2O$), potassium chlorate ($KClO_3$), potassium chloride (KCl), sodium chloride ($NaCl$), commercial iron chloride, potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$), sodium malate ($Na_2C_4H_4O_5$), ammonium malate ($Na_2C_4H_4O_5$), sodium chinate ($NaC_7H_{11}O_7.2H_2O$), sodium butyrate ($NaCOC_4H_7O_2$), phosphoric acid (H_3PO_4), and alcohol exerted negative chemiotropic action on all species studied at all dilutions tested.

[2] *Ward, H. Marshall.* On some relations between host and parasite in certain epidemic diseases of plants (Proc. Royal Soc., 47, pp. 393–443).

[3] *Büsgen*, l. c. Ueber einige Eigenschaften der Keimlinge parasitischer Pilze.

in immediate contact with copper particles would be very likely to effect solution of at least a little copper.[1] Reinhardt[2] has shown that the growth of hyphæ takes place only at the extreme tips, so the chances are that the tips of the hypha, the part most likely to encounter copper, would be most sensitive to the effect. Again, in many parasitic fungi the delicate tube which, growing out from a contact organ, pierces the epidermal wall, must exert some solvent action on the cuticle and on cellulose. If, as Millardet and Gayon[3] claim, the cuticle absorbs copper salts greedily from solutions and retains them with great tenacity, the delicate penetration tube in piercing the cuticle would be compelled to dissolve a certain though small quantity of the poison.

(6) The germ tube may be so much injured soon after germination as to cease growing before attempting to effect an entrance into the host plant, or may be killed outright soon after appearing. In either case the copper in solution would be the active agent. This is very probably a common mode in which the bordeaux mixture and other copper fungicides act. The accumulation of copper in the fungus probably continues until no further growth is possible; then if more is absorbed death soon results. Even if not killed outright by the copper, the germ tubes would be little fitted to withstand the drying likely to occur within a few hours.

(7) The effect of the copper contained in bordeaux mixture may be exerted at a later stage of development of the fungi. Although in most cases the action of the copper doubtless takes place on the spores, zoospores, or young germ tubes, yet with species that may spread by means of mycelium[4] (e. g. Sclerotinia) it may occur at any stage of development.

(8) There is a possibility that the presence of a thick coating of copper salts might impede the fruiting of a fungus already within the

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[1] *Frank, B., and Krüger, F.*, Ueber den Reiz welchen die Behandlungen mit Kupfer auf die Kartoffel-Pflanze hervorbringt (Ber. d. Deutsch. Bot. Ges., 12, Heft I, 1894, p. 10) state that spores of Peronosporaceæ and other fungi were killed by soaking twenty-four hours in bordeaux mixture several weeks old, but were not killed by the same treatment with the clear liquid obtained by filtering the mixture. This clear liquid showed no copper with chemical tests. They concluded that the presence of the insoluble copper hydrate is necessary to kill the spores. It is not, however, stated whether the liquid was free from calcium hydroxide and thus able to dissolve any copper. Further, the filter may have absorbed the small quantities of copper present, or the secretions of the fungi may have dissolved copper. The conditions were so very different from those occurring in practice that the experiment as reported seems of little value.

[2] *Reinhardt*, l. c. Das Wachstum der Pilzhyphen.

[3] *Millardet et Gayon.* Recherches nouvelles sur l'action que les préparations cuivreuses exercent sur la Peronospora de la vigne (Jour. d'Agr. prat., 51e ann., T. I, No. 4, 1887, pp. 27–129; No. 5, pp. 156–162).

[4] Such as for instance the *Selerotinia libertiana*, causing the disease of chicory (see *Prillieux*, Une maladie de la barbe de capucin, Jour. d'Agr. prat., 57e ann., II, No. 11, 1893, pp. 387–388) and other Sclerotiniæ.

tissues of the host. This is, however, improbable. Neither is there any adequate support for the views held by some writers, that copper absorbed into the tissues of the host either through the root[1] or through the leaves,[2] may hinder or prevent the development of fungi after they effect an entrance.

HYPOTHESIS AS TO THE NATURE OF THE ACTION OF COPPER IN PREVENTING ATTACKS OF PARASITIC FUNGI.

Loew, in his general treatise[3] on poisoning, includes copper, with other heavy metals, among the general poisons, i. e., those which in moderate concentration are fatal to all living beings. He considers the action to be one of salt production, and thinks that the heavy metals probably attack the living protoplasm, very likely replacing hydrogen in the amido acid groups which probably occur in proteid matter. Silver and mercury especially are said to replace very readily hydrogen atoms of the amido group and may owe their unusually poisonous action to this property. It is quite probable that the action of copper would be very similar to that of silver or mercury, as it is closely related to them chemically. The hydrogen in carboxyl is also capable of being replaced by the heavy metals.[4] It may be that the action of copper on parasitic fungi is similar to the curious oligodynamic action on Spirogyra observed by Nägeli. He found that metallic copper placed in contact with distilled water caused either oligodynamic phenomena or phenomena resembling natural death, but never chemical poisoning. Nägeli considers the oligodynamic action a very different one from chemical poisoning or from that of very dilute solution in causing natural death, and states that "the oligodynamic reaction consists in a specific sensitiveness of the green plasma." As fungi do not contain trophoplasts, it could not be assumed that any such specific action takes place in them. The subject is one well worthy of further study and promises results of the greatest interest, not only in a practical way, but also from a physiological standpoint.

[1] *Schloesing* in Soc. Nat. d'Agr. France, Séance Nov. 11, 1885 (reported by *Pradel, E. C.*, Jour. d'Agr. prat., 49e ann., T. II, No. 471, 1885, p. 747; also *Pichi*, Alcuni esperimenti fisio-patologiche sulla vite in relazione al parasitismo della peronospora. Nuovo giornale bot. italiano, 23, No. 2, 1891, p. 361).

Millardet et Gayon. Recherches nouvelles sur l'action que les préparations cuivreuses exercent sur le Peronospora de la vigne (Jour. d'Agr. prat., 51e ann., T. I, No. 5, 1887, pp. 160, 161).

[3] *Loew, Dr. O.* Ein natürliches System der Giftwirkungen (München, Wolff, and Lüneburg, 1893, 8°, 8+136).

[4] *Loew, l. c.*, pp. 14–38. On pp. 31, 32 he says: "The protein matters resemble most the amido acids in their chemical character. They can combine with either acids or bases and form salts like compounds. If now this occurs in the protein matters of living protoplasts, it may occasion derangements that may cause death."

SUMMARY.

(1) Freshly made bordeaux mixture consists essentially of cupric hydroxide and calcium sulphate in solid form, suspended in a nearly or quite saturated solution of calcium sulphate and calcium hydroxide in water.

(2) The most soluble constituent is calcium sulphate, one part of which dissolves at ordinary temperatures in from 450 to 550 parts of water.

(3) Calcium hydroxide is also present in solution, in considerable amounts, in the mixture as ordinarily made, with a slight excess of lime. This substance is soluble at ordinary temperatures in from 775 to 825 parts of water.

(4) Probably no cupric hydroxide or any other copper salt is present in solution in the properly made mixture, since it is insoluble in water containing calcium hydroxide.

(5) The cupric hydrate in freshly made bordeaux mixture is in the form of precipitation membranes. These membranes are thin, but of great extent and probably of a colloid nature, containing large amounts of imbibed water. This causes the precipitate to be very bulky and to settle very slowly.

(6) On standing, especially if the mixture is agitated occasionally, the precipitation membranes disappear, being used up in the formation of crystals of copper hydroxide. These crystals, often in the form of sphærocrystals, settle very rapidly after the liquid is agitated.

(7) When the mixture is made the calcium sulphate is thrown down in the form of small amorphous granules. After standing, it often appears as small twin crystals.

(8) The character of the cuticle of the plant sprayed, whether easily wetted or not, and also the fineness of the spray, are factors having much influence on the adherence of the mixture.

(9) As the water in a drop of the mixture evaporates, calcium sulphate soon begins to crystallize out and cements the precipitates together and to the leaf.

(10) On exposure to the air and rains, the calcium hydroxide present is gradually changed to calcium carbonate by absorbing CO_2 from the atmosphere. This calcium carbonate, being very hard and very difficultly soluble, aids in cementing the precipitates to the leaf.

(11) The colloid precipitation membranes in the fresh mixture may on drying attach themselves firmly to the leaf surface.

(12) The presence of difficultly soluble cementing substances and the absence of readily soluble constituents are the causes of the great adhesiveness of bordeaux mixture.

(13) The cupric hydroxide is not dissolved by rain or dew till all the calcium hydroxide originally present has been changed to carbonate.

(14) The neutralization of calcium hydroxide is greatly favored by soft rains and dew, and even by very damp air.

(15) The CO_2 of the air, which dissolves in water at ordinary temperatures at the rate of about one-third to three-fourths of a milligram per liter, probably plays an important part in dissolving copper.

(16) Ammonium carbonate, nitrate, and nitrite present in small amounts in rain water doubtless dissolve slight amounts of copper, probably by forming soluble cuproammonium salts.

(17) Small amounts of cupric hydroxide may dissolve in water in the absence of calcium hydroxide.

(18) Substances absorbed by water from the cells of the host plant, such as sugar, may serve to dissolve copper.

(19) Substances secreted by the fungus itself may aid in effecting solution of small quantities of copper.

(20) The fungus may, by absorbing the slight amount of copper in solution, make it possible for the water to take up a fresh quantity, which is in turn absorbed, making it possible for a considerable amount of copper to be absorbed by the fungus, even though the water contains but slight quantities at any one time.

(21) It was found by Benedict Prevost that 10 or 12 milligrams of a fine powder of copper in 150 cubic centimeters of water prevented the germination of *Tilletia tritici*, as did also plates of cleaned and uncleaned brass or copper, copper coins, and bell metal. Where ordinary well water was used, the spores of this fungus failed to germinate in a solution of 1 part of copper sulphate to 250,000 of this water at a temperature of 6¼° to 7½° C., but they sometimes germinated in a solution of 1 part copper sulphate to 200,000 of water. Germination was sensibly retarded in a solution of 1 to 600,000, or even 1 to 1,000,000, but was not prevented by soaking some hours in a solution of 1 to 10,000, followed by washing in water. With distilled water, 1 part copper sulphate to 200,000 parts water, at a high temperature, had almost no retarding effect on the germination of the spores, but in a solution of 1 to 100,000 germination was entirely prevented. In a solution made by treating copper with vinegar and diluting with water until it contained 1 part to 30,000, the germinative power of the spores soaked in it four hours, at 22½° to 25° C., was destroyed.

(22) The researches of Millardet and Gayon showed that the zoospores of downy mildew were killed in a short time in a solution of 2 or 3 parts copper sulphate to 10,000,000 of water, but that it required 1 part of lime to 10,000 of water to accomplish the same result.

(23) Dufour's experiments showed that *Claviceps purpurea*, *Fusicladium pyrinum*, Pleospora, and Phragmidium germinated in a solution of 1 part copper to 1,000,000 parts water.

(24) Experiments carried on by Wüthrich showed that the conidia of *Phytophthora infestans* would not germinate in solutions of 1 part copper sulphate to 8,000 parts water or 1 part copper sulphate to 8,000 parts malt extract, and that the motion of the free zoospores of this fungus instantly ceased in a solution of 1 part copper sulphate to 8,000

parts water. The conidia of *Plasmopara viticola* would not germinate in 1 part copper sulphate to 80,000 parts water, and the motion of the free zoospores stopped in one minute in a solution of 1 to 80,000. In the case of *Ustilago avenæ* there was no germination of spores in a solution of 1 part copper sulphate to 8,000 parts water or 1 part copper sulphate to 80 parts malt extract. In a strength of 1 part copper sulphate to 800 parts water there was no germination of the uredospores of *Puccinia graminis*, and no germination of the æcidiospores in a solution of 1 to 8,000. The germination of the conidia of *Claviceps purpurea* was prevented in a solution of 1 to 8,000.

(25) In Carleton's experiments with *Puccinia rubigo-vera* and *P. graminis* 1 part of copper sulphate or nitrate to 1,000 parts water usually prevented almost all germination of the uredospores.

(26) Some of the mold fungi are remarkably indifferent to the effects of copper sulphate. Manasein claims that solutions of this salt must be as strong as one-fourth of 1 per cent to produce any appreciable effect on these fungi.

(27) Nägeli found that the cells of Spirogyra on which he experimented were killed in four different ways, according to the strength of the toxic agent used: (*a*) Very strong solutions caused physical injury, extreme plasmolysis, etc.; (*b*) weaker solutions caused what he calls chemical poisonous action; (*c*) still weaker solutions caused oligodynamic action; and (*d*) yet weaker solutions caused changes resembling those of natural death. Most poisonous solutions could produce all the kinds of poisoning described.

(28) Pure distilled water, when placed in contact with copper, silver, or mercury, which were found to be exceedingly poisonous, became capable of producing strong oligodynamic effects. Nägeli showed that if metallic copper, mercury, difficultly soluble copper, or silver compounds were left in pure water in glass vessels, after a time the glass acquired the same properties and the vessels would speedily render fresh water poisonous when placed in them.

(29) Large surfaces and various insoluble substances often neutralized this poisonous action of water, but chemically related molecular soluble substances possess no such power. Dilute hydrochloric and nitric acids, and also prolonged boiling in large quantities of water, destroyed this poisonous effect on the glass vessels.

(30) The following suggestions are made in regard to the possible action of copper on fungi: (*a*) The spores may be prevented from germinating by inhibitory action; (*b*) the protoplasmic content of the spores may be killed outright in a short time before germination has commenced; (*c*) through negative chemiotropic action of the copper hydroxide the germ tube may be prevented from entering the plant; (*d*) the germ tube may be so weakened by copper in solution as to be unable to enter the host plant; (*e*) the germination tubes may be prevented from

growing or be killed only upon contact with solid particles of copper or its compounds, or with the cuticle or other parts of the host impregnated with copper; (*f*) the germ tube may be so much injured soon after germination as to cease growing before attempting to effect an entrance into the host plant, or may be killed outright soon after appearing; (*g*) the effect of the copper contained in bordeaux mixture may be exerted at a later stage of development of the fungi; (*h*) the presence of a thick coating of copper salts might impede the fruiting of a fungus already within the tissues of the host plant.